Isolated Loss of PMS2 Expression in Colorectal and Endometrial Tumors Explained with Paired Tumor and Germline Testing

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Background: Candidates for Lynch syndrome genetic testing are frequently identified by abnormal microsatellite instability (MSI) and/or mismatch repair (MMR) protein immunohistochemistry (IHC) on colorectal and endometrial tumors. PMS2 is one member of the DNA mismatch repair complex, and its expression is typically lost in tumors that also exhibit loss of MLH1 expression. Rarely, isolated loss of PMS2 expression is observed. Recent data suggest isolated loss of PMS2 (IL-PMS2) may be the result of germline *PMS2* mutations, germline *MLH1* mutations, or *MLH1* promoter hypermethylation. This study aimed to describe the results of paired tumor and germline DNA testing of the MMR genes for individuals with tumors demonstrating IL-PMS2 at one laboratory.

Methods: A retrospective analysis was performed on data from colorectal and endometrial cancer patients with IL- PMS2 identified by IHC analysis from external laboratories. Patients underwent paired tumor and germline MMR gene analyses and *MLH1* promoter hypermethylation studies between 12/05/16-11/24/17. Results of next generation DNA sequencing and deletion/duplication analyses of the MMR genes and *EPCAM* (deletion/duplication only) were assessed.

Results: Of 34 patients with colorectal or endometrial tumors demonstrating IL-PMS2, ten (29%) carried germline *PMS2* mutations and two (6%) carried germline *MLH1* mutations/likely pathogenic variants (VLPs). Nine patients (26%) had tumors demonstrating *MLH1* promoter hypermethylation. Two cases (6%) demonstrated one somatic *MLH1* mutation/VLP in the presence of *MLH1* copy-neutral loss of heterozygosity (CN-LOH) (Table 1). The remaining 11 cases (33%) were not explained by a germline mutation, somatic inactivation of *MLH1* and/or *PMS2,* or *MLH1* promoter hypermethylation (Table 2).

Conclusion: In this cohort, 67% of tumors demonstrating IL-PMS2 were explained by germline *PMS2* or *MLH1* mutations, *MLH1* promoter hypermethylation, or MMR deficiency due to one somatic *MLH1* mutation in the presence of CN-LOH. These data support the importance of adding comprehensive paired tumor and germline MMR gene analyses to the Lynch syndrome testing algorithm which will allow clinicians to further distinguish between sporadic tumors and Lynch syndrome.

Table 1 Informative Cases Explaining IL-PMS2

Description of Test Result	n (%)	Additional Molecular Characteristics	Additional Clinical Characteristics	Diagnosis
Germline *PMS2* mutation	10 (29%)	8/10 had second somatic *PMS2* hit	3/10 met Amsterdam II criteria (AM) 5 endometrial cancers (EC), mean 70 years 5 colorectal cancers (CRC), mean 67 years	Lynch syndrome
Germline *MLH1* mutation/VLP	2 (6%)	2/2 had second somatic *MLH1* hit	1/2 met AM 2 CRC, 32 and 44 years	Lynch syndrome
MLH1 promoter hypermethylation	9 (26%)	1/9 demonstrated 2 somatic *PMS2* mutations	0/9 met AM 4 EC, mean 60 years 5 CRC, mean 66 years	IHC likely due to somatic changes
1 somatic *MLH1* mutation/VLP and CN-LOH[a]	2 (6%)		0/2 met AM 2 CRC, 58 and 66 years	IHC likely due to somatic changes
	Total: 23 (67%)			

[a]One tumor showed equivocal (10%) MLH1 staining.

Table 2 Uninformative Cases

Molecular Test Results[a]	n (%)	Additional Molecular Characteristics	Additional Clinical Characteristics
Germline *PMS2* VUS	2 (6%)	1 with somatic *PMS2* mutation and somatic *PMS2* VUS	0/2 met AM 2 CRC, 40 and 61 years
Germline *MSH6* VUS	1 (3%)	Also had 1 somatic *MLH1* VUS	Did not meet AM CRC 54 years and breast cancer 45 years
1 somatic *PMS2* mutation	4 (12%)	1 with 2 somatic *PMS2* VUSs 1 with 1 somatic *MLH1* mutation	0/4 met AM 4 EC, mean 59 years
1 somatic *MLH1* mutation	1 (3%)		Did not meet AM CRC 36 years
1 somatic *MSH2* mutation	1 (3%)	2 somatic *PMS2* VUSs and somatic *MLH1* VUS	Met AM EC 66 years
1 somatic *MLH1* VUS[b]	1 (3%)		Did not meet AM EC 58 years
No germline or somatic hits	1 (3%)	MSS	Did not meet AM CRC 43 years
	Total: 11 (33%)		

[a]Additional somatic mutations and/or variants not believed to be associated with the IHC results were excluded.

[b] *MLH1* promoter hypermethylation studies were not performed for this case.